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# Energetics of Bacterial Growth: Balance of Anabolic and Catabolic Reactions

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## INTRODUCTION

Since Leeuwenhoek first observed bacteria more than 250 years ago, microbiologists have made extraordinary progress in studying this extremely diverse group of living organisms. The processes of energy source degradation, ATP formation, monomer synthesis, macromolecular polymerization, DNA replication, and cell duplication are surprisingly well understood. However, despite the abundance of information on the details of bacterial metabolism, there has been little quantitative information regarding the thermodynamics and kinetics of bacterial growth.

The growth strategies of bacteria are sometimes manifested by rapid cell division, but the mathematics of exponential growth readily illustrate the point that bacteria cannot grow rapidly for long periods. If a bacterium had an intracellular volume of 1 $\mu m^3$ and a doubling time of 20 min, this single cell would generate a volume of protoplasm $2.2 \times 10^{25}$ $m^3$ in 48 h. Because the volume of the Earth is only $1.1 \times 10^{21}$ $m^3$, it is clear that "in the life of a bacterium, any number of essential nutrients can and do often become limiting" (72).

When bacteria are grown on agar plates, quantitative aspects of biomass formation are completely disregarded, and in many cases the growth assessment of broth cultures has been limited to semiquantitative scorings (e.g., +++ to +/−) (118). With the advent of continuous-culture techniques in the 1950s, microbiologists were able to grow bacteria under defined growth rates and steady-state conditions, but many continuous-culture experiments were simply an exercise of feeding and weighing bacteria. The distinction between energy and carbon source utilization, precise estimations of ATP generation, and potential variations in bacterial composition were often overlooked.

Standard textbooks of biochemistry have often promoted the idea that "cells are capable of regulating their metabolic reactions and the biosynthesis of their enzymes to achieve maximum efficiency and economy" (55), and microbiologists have generally assumed that the "yield of cells is directly proportional to the amount of ATP produced" (8). This assumption of a strict coupling between anabolism and catabolism is contradicted by the observation that "resting-cell suspensions" can utilize energy sources in the complete absence of growth and by the fact that the correlation between ATP and biomass

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TABLE 1. ATP requirement for the formation of bacterial cells from glucose[a]

| Macromolecule | % Dry wt | ATP requirement (mmol/g of macromolecule) | |
|---|---|---|---|
| | | With amino acids | Without amino acids |
| Protein | 52.2 | | |
| Amino acid formation | | 0.0 | 1.4 |
| Polymerization | | 19.0 | 19.0 |
| Polysaccharide | 16.6 | | |
| G6P[b] formation | | 1.0 | 1.0 |
| Polymerization | | 1.0 | 1.0 |
| RNA | 15.7 | | |
| Nucleoside formation | | 1.5 | 1.5 |
| Polymerization | | 0.9 | 0.9 |
| DNA | 3.2 | | |
| Nucleoside formation | | 0.4 | 0.4 |
| Polymerization | | 0.2 | 0.4 |
| Lipid | 9.4 | 0.1 | 0.1 |
| Other functions | | | |
| mRNA turnover | | 1.4 | 1.4 |
| Transport of ammonium ions | | 0.0 | 4.2 |
| Transport of amino acids | | 4.8 | 0.0 |
| Transport of potassium | | 0.2 | 0.2 |
| Transport of phosphate | | 0.8 | 0.8 |
| Total | | 31.3 | 32.3 |
| $Y_{ATP}$ (g of cells/mol of ATP) | | 32 | 31 |

[a] Modified from reference 107.
[b] G6P, glucose 6-phosphate.

formation is often very poor. Some of the variation in growth efficiency can be explained by maintenance energy expenditures, but bacteria have other mechanisms of nongrowth energy dissipation.

## $Y_{ATP}$ VALUES

When Monod (68) studied the growth of *Bacillus subtilis*, *Escherichia coli*, and *Salmonella typhimurium* batch cultures, the dry weight of the organisms was directly proportional to the amount of energy source added, but there was no estimation of ATP production from carbohydrate fermentation. *Enterococcus* (*Streptococcus*) *faecalis* produced more biomass from glucose fermentation than did *Lactobacillus mesenteroides* (25), and subsequent work showed that these two bacteria used different pathways of fermentation and produced different amounts of ATP/glucose (43). By the late 1950s, bacteriologists had generally accepted the idea that cell yield was roughly equivalent to energy yield (102, 104).

In 1960, Bauchop and Elsden (5) studied the growth of several anaerobic bacteria and correlated biomass production with ATP availability ($Y_{ATP}$). They obtained an average value of 10.5 g of cells per mol of ATP, but the range was actually 8.3 to 12.6 g/mol. Despite the more than 50% variation, the 10.5 value for $Y_{ATP}$ was treated as a biological constant (8, 37, 47, 105). Cellular dry weight and $Y_{ATP}$ have even been used as a method of estimating the ATP production of a suspected catabolic scheme (47, 105).

By the 1970s, the notion of a constant $Y_{ATP}$, however, was being questioned. A review of the literature indicated that there was at least a fivefold range in $Y_{ATP}$ values (107), and Stouthamer's calculations (Table 1) indicated that $Y_{ATP}$ should be threefold higher than the value derived by Bauchop and Elsden (32 versus 10.5 g of cells per mol of ATP). These inconsistencies led Tempest and Neijssel (118) to conclude that "yield values *per se* are not readily interpretable in precise bioenergetic and/or physiological terms, and, unless treated with considerable circumspection, they may lead to the formation of concepts that are at best dubious."

TABLE 2. Free energy change of various phosphate transfer reactions[a,b]

| Phosphoryl donor | $\Delta G'$ (kcal/mol)[b] |
|---|---|
| Phosphoenolpyruvate | −12.8 |
| 1,3-Diphosphoglycerate | −11.8 |
| β-L-Aspartyl phosphate | −11.5 |
| Acetyl phosphate | −10.1 |
| ATP | −7.6 |
| $PP_i$ | −6.6 |
| ADP | −6.4 |
| Galactose 1-phosphate | −5.0 |
| Glucose 1-phosphate | −5.0 |
| 2-Phosphoglycerate | −4.2 |
| Fructose 6-phosphate | −3.8 |
| Glucose 6-phosphate | −3.3 |
| 3-Phosphoglycerate | −3.1 |
| Fructose 1-phosphate | −3.1 |
| Glycerol 1-phosphate | −2.3 |

[a] Modified from reference 128.
[b] 1 kcal/mol = 4.184 kJ/mol.

## Factors Affecting $Y_{ATP}$ Determinations

**Estimation of ATP production.** The role of phosphate esters in energy transduction of living cells was first recognized by Harden and Young in 1906 (41), but it was not until the 1940s that the significance of phosphate esters was more fully appreciated. Lipmann (56) used the term "energy rich" to describe ATP and other phosphorylated intermediates, and with time, phosphate bond formation and breakage were recognized as means of energy exchange. ATP is often assigned a standard free energy ($\Delta G$) value of −7.6 kcal/mol (−31.8 kJ/mol) (Table 2), but as Nicholls (77) noted, it is the displacement of the mass action ratio "from equilibrium which defines the capacity of the reactants to do work, rather than the attributes of a single component." Since it is often difficult to determine the mass action ratio under physiological conditions, the $\Delta G$ values are usually little more than conjecture.

The study of bioenergetics has also been confounded by the fact that cells can use two distinctly different methods of ATP generation. Soluble phosphate transferases (kinases) have a well-defined ATP stoichiometry (Table 2), but ATP production from chemiosmotic mechanisms (56, 66) has been "a lively topic of debate" that has yielded little lasting consensus (40). Some textbooks of microbiology still indicate that glucose oxidation involves three coupling sites, with the complete oxidation of glucose producing 38 ATP mol per mol (8), but bacteria do not usually have three phosphorylation sites (37, 39). In *E. coli* there are several pathways of respiration, and the physiological mechanisms regulating the flow of electrons are still not well understood (39).

Mitchell and Moyle (67), by relating the standard free energy of ATP hydrolysis ($\Delta G'p$) to the proton motive force ($\Delta p$), indicated that the proton stoichiometry of the mitochondrial membrane-bound ATPase was approximately 2, but similar measurements with bacteria indicated that the stoichiometry could be 3, or even greater (59, 60, 82). Each of these estimates assumed an intracellular magnesium concentration of approximately 10 mM, an equilibrium between the bulk phase $\Delta p$ and localized charge movement through the membrane-bound

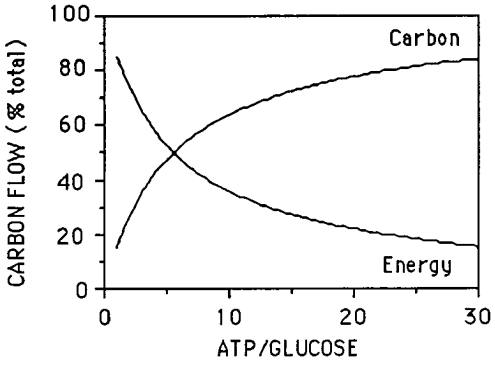


FIG. 1. Effect of ATP on the amount of carbon that would be used as an energy source versus the amount that would be incorporated into cell carbon. The relationships are based on a $Y_{ATP}$ of 32 g of cells per mol of ATP for the growth of bacteria in minimal media. Carbon and energy were calculated from the simultaneous equations: carbon + energy = 1 and 1/32 g of cells per mol of ATP + ATP/energy source. Energy requirements were not corrected for maintenance energy.

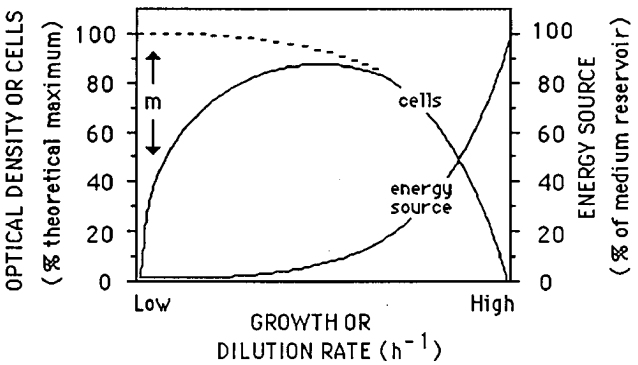


FIG. 2. Growth of a bacterium in an energy-limited chemostat under steady-state conditions. As the dilution rate increases, the concentration of energy source remaining in the chemostat vessel increases until it is equal to the concentration of energy source in the medium reservoir. As the energy source accumulates in the chemostat vessel, the cell density declines and the culture washes out when the concentration of energy source in the chemostat vessel equals the reservoir ($\mu_{max}$). At low dilution rates, the cell density also declines, because a larger fraction of the energy source utilization must be devoted to maintenance functions. The dotted line shows the cell density which would be obtained if there were no maintenance energy.

ATPase, and a negligible contribution of passive proton leaks (46, 53, 133). More direct estimates that were based on ΔpH relaxation (84) and heat dissipation (6) indicated that the proton/ATP ratio was only 1.9. Given these considerations, it not surprising that there is large variations in the $Y_{ATP}$ of aerobes.

**Energy source utilization for carbon.** Bacterial-cell yields are often calculated from energy source depletion, but such simple estimates of energy source utilization do not account for the incorporation of energy source into cell material. The overestimation of ATP production is dependent on the amount of ATP that the energy source generates. If the ATP production from the catabolic scheme is low, most of the energy source will be used for energy, even if other carbon sources are not available. As the ATP production increases, however, the fraction of energy source that is used for carbon can be very significant. On the basis of growth in a minimal medium and a $Y_{ATP}$ value of 32 g of cells per mol of ATP, as much as 90% of the energy source could be used for cell carbon (Fig. 1). Bauchop and Elsden (5) indicated that *Enterobacter faecalis* diverted only 4% of the energy source to cell carbon, but other researchers have not determined the source of cell carbon (38).

**Changes in cell composition.** In the 1970s, Stouthamer (107) calculated the amount of ATP which would be needed to produce bacterial biomass and based these calculations on a cell composition that was typical of *E. coli* (see Table 1). These calculations illustrated at least three major points. First, polymerization reactions, and in particular protein synthesis, are clearly the most demanding steps of biomass formation. Second, monomer biosynthesis per se (amino acids, nucleotides, etc.) utilizes only a small fraction of the total ATP. Third, transport of carbon sources and osmolytes accounts for less than one-quarter of the total ATP requirement.

RNA and polysaccharide are the components of the bacterial cell that are most likely to change. When *E. coli* increases its growth rate, there is a commensurate decrease in protein levels (57), but even a 2.5-fold change in RNA would cause less than a 7% variation in $Y_{ATP}$. The energetic difference between polysaccharide and protein is greater, but once again it would take a fairly large increase in polysaccharide to affect $Y_{ATP}$. When the ruminal bacterium *Prevotella ruminicola* was grown under nitrogen limitation, polysaccharide was 1.5 times greater than protein but the $Y_{ATP}$ was only 25% higher than for carbon-limited cells (94).

**Maintenance energy.** The concept of $Y_{ATP}$ assumes that all of the energy from catabolism can be used for growth; however, bacteria also expend energy on functions that are not directly growth related. Direct estimates of maintenance were confounded by the fact that until recently, microbiologists did not have sensitive equipment for measuring very low rates of catabolism (92). Additional confusion arose from the observation that the maintenance rate of growing cells is not always the same as the endogenous metabolic rate of cells that are starving (see the section on maintenance versus endogenous metabolism below).

In the 1920s, Buchanan and Fulmer (10) noted that low levels of energy sources were not effective in subculturing bacteria, even if the transfer interval was short and suggested that bacteria needed some energy to "maintain" the cells. Monod (68) considered the possibility of maintenance energy in his classic treatise on bacterial growth, but the approach of estimating maintenance from the negative intercept of glucose concentration versus optical density indicated that the maintenance energies of *E. coli* and *B. subtilis* were essentially zero. In the early 1960s, McGrew and Mallette (65) tried to estimate bacterial maintenance energy by determining the amount of glucose which would be needed to prevent a decrease in optical density, but once again this approach did not provide a clear-cut distinction between the maintenance energy of growing cells and endogenous metabolism of starving cultures.

Duclaux (28) provided a mathematical derivation of maintenance in 1898, but there were few data with which to test his model. With the advent of continuous-culture techniques and the growth of bacteria at defined and submaximal growth rates (69, 79), the estimation of maintenance energy became a more straightforward exercise. Since maintenance is a function that detracts from growth, the contribution of maintenance is more pronounced when the growth rate is low (Fig. 2). Herbert et al. (45) conceptualized maintenance energy as "negative growth," and this theme was continued in the maintenance derivation of Marr et al. (63). According to Marr et al. (63), maintenance energy can be described by a negative growth rate constant ($a$),

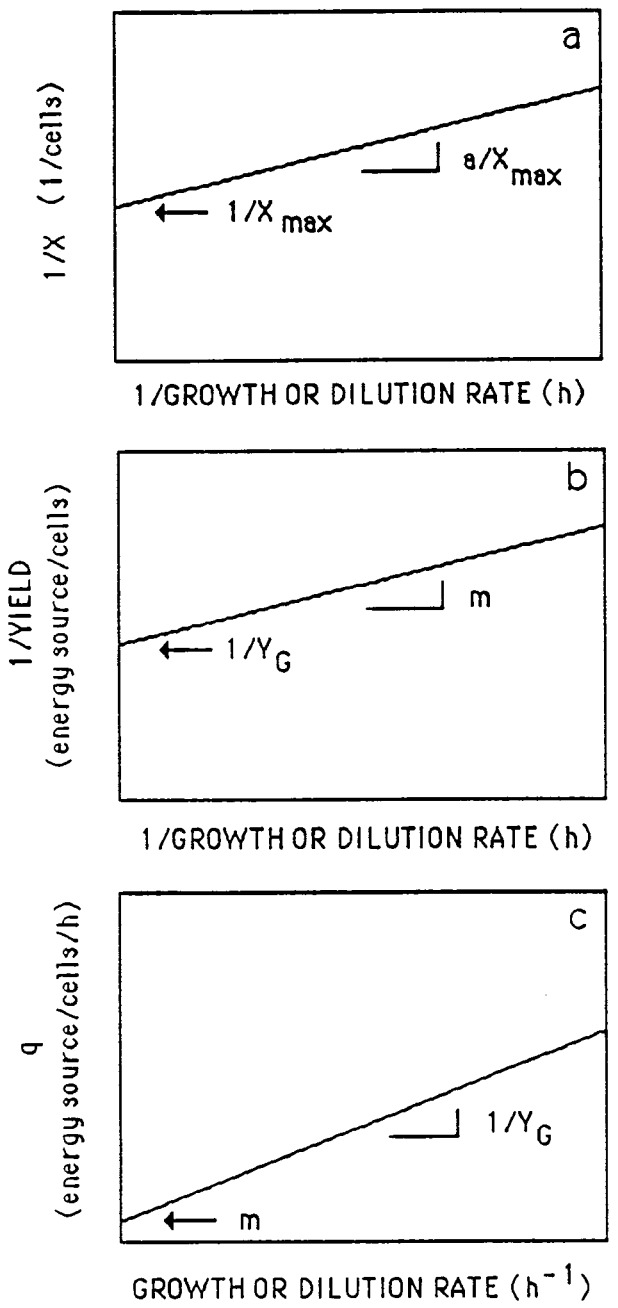


FIG. 3. Effect of maintenance rate (*a*) or the maintenance coefficient (*m*) on the yield of a bacterium grown in an energy-limited chemostat. (a) Derivation of Marr et al. (63); (b) derivation of Pirt (85); (c) derivation of Tempest and Neijssel (118). $Y_G$ is defined as the theoretical maximum growth yield.

and the total rate of substrate utilization for growth $[(-dS/dt) \cdot Y]$ can then be partitioned into growth ($\mu x$) and negative growth ($ax$):

$$(-dS/dt) \cdot Y = \mu x + ax$$

From these assumptions, $a$ causes a decrease in the theoretical maximum growth rate of the organism, and $\mu_{max}$ can be envisioned as the growth rate that would be obtained if there were no maintenance:

$$\mu_{max} = \mu + a$$

If one defines $x_{max}$ as the theoretical maximum cell mass (the cell mass produced if there were no maintenance), one can derive the equation of a straight line (Fig. 3a):

$$\frac{1}{x} = \frac{a}{x_{max}} \cdot \frac{1}{\mu} + \frac{1}{x_{max}}$$

A few years later, Pirt (85) indicated that the "negative growth" concept of Marr et al. (63) was "artificial and indirect" and proposed a less hypothetical approach. The negative growth rate concept was circumvented by describing maintenance by a "coefficient" ($m$) that described the amount of energy needed to maintain cells for a given period (energy/cells/time). In Pirt's derivation, maintenance has no direct effect on growth rate, but the yield is decreased. On the basis of the assumption that $Y$ is the actual yield of bacteria (grams of bacteria per gram of energy source) and $Y_G$ is the theoretical maximum yield ($Y$ if there were no maintenance), total energy utilization ($\mu x/Y$) can be partitioned into maintenance ($mx$) and true growth ($\mu x/Y_G$):

$$\mu x/Y = mx + \mu x/Y_G$$

Using the same type of algebraic transformations as Marr et al. (63), Pirt succeeded in deriving another straight-line equation (Fig. 3b):

$$\frac{1}{Y} = \frac{m}{\mu} + \frac{1}{Y_G} \quad (1)$$

The two maintenance parameters, $m$ and $a$, are related by

$$m = a/Y_G$$

More recently, Tempest and colleagues (75, 117) algebraically modified the derivation of Pirt (equation 1) to get another linear relationship (Fig. 3c). Assuming that the specific rate of energy source consumption $q = (1/Y)\mu$ and $1/Y = q/\mu$,

$$\frac{q}{\mu} = \frac{m}{\mu} + \frac{1}{Y_G}$$

Multiplying by $\mu$,

$$q = m + \mu \cdot 1/Y_G$$

The plots used by Pirt and Tempest both define $m$ as a specific coefficient, but the experimental error of the plots is partitioned differently. In the Pirt plot (Fig. 3b), the error is primarily in the slope, whereas the error of the Tempest plot (Fig. 3c) is primarily in the intercept. Since the intercept of the Tempest plot is usually small, the data always look better.

The linearity of maintenance plots is based on the assumptions that the ATP production per unit of energy source does not change, cell composition remains the same, and maintenance is a strictly mass- and time-dependent function. Pirt (85) noted that the maintenance plot of *Selenomonas ruminantium* was not linear and indicated that the change in yield could not readily be attributed to maintenance. Later work showed that *S. ruminantium* switches fermentation end products and gets more molecules of ATP per molecule of glucose when the growth rate is low (97). Pirt plots of the amino acid-fermenting bacterium *Clostridium sticklandii* (124) indicated that arginine-limited cells had twice as high a maintenance coefficient (based on ATP) as did lysine-limited cells, but this difference was caused by transport rather than maintenance per se. *C. sticklandii* always used facilitated diffusion to take up lysine, but arginine was taken up by either sodium symport (active transport) or facilitated diffusion. When the dilution rate and argi-

TABLE 3. $Y_{ATP/MAX}$ of various microorganisms grown in glucose-limited continuous culture[a]

| Microorganism | $Y_{ATP/MAX}$[b] |
|---|---|
| *Lactobacillus casei* | 24.3 |
| *Aerobacter aerogenes* | 14.0 |
| *Escherichia coli* | 10.3 |
| *Saccharomyces cerevisiae* | 13.0 |
| *Candida parapsilosis* | 12.5 |

[a] Modified from reference 107.
[b] Grams of cells per mole of ATP.

nine concentration decreased, *C. sticklandii* gradually switched from facilitated diffusion to sodium symport. The increased cost of arginine transport at low dilution rates in continuous culture caused a decrease in cell yield and an increase in the slope of the Pirt plot.

In the mathematical derivations, maintenance is loosely defined as any diversion of energy from "growth" to "nongrowth" reactions, but this definition gives little mechanistic insight into the nature of maintenance functions. In many cases, the difference between maintenance and growth is dictated by nothing more than the difference between net and gross. For example, protein synthesis is a growth-related function, but protein turnover (degradation and resynthesis) is a maintenance expenditure.

Radiolabeling experiments indicated that the rate of protein turnover in exponentially growing *E. coli* cells ranged from 0.5 to 2.5%/h and that RNA turnover paralleled the breakdown of protein (61). During stationary phase, protein turnover was higher (5% for *E. coli* and 8% for *Bacillus cereus* [61]), but even these rates cannot account for all of the maintenance in *E. coli*. On the basis of a cell composition of 0.5 g of protein per g of cells, 4 ATP equivalents per amino acid polymerized, an average molecular mass for an amino acid of 100 Da, and a maximum ATP production of 24 ATP per glucose, the glucose consumption rate needed to sustain a protein turnover rate of 5%/h would be 0.04 mmol of glucose per g of cells per h. The maintenance rate of *E. coli* is 0.31 mmol of glucose per g of cells per h (85).

Ingraham et al. (47) listed the "accumulation of substrates to a higher concentration" as a maintenance function, but Stouthamer indicated that most transport functions are growth related (Table 1). Once again, the difference between growth and maintenance is related to turnover. It was originally assumed that bacterial membranes were perfect insulators (66), but bacterial membranes have an inherent or passive permeability to most ions (59, 120). Ion fluxes across the membranes of growing bacteria have not been measured directly, but ion turnover is likely to be a very significant component of maintenance. Because the flagella of bacteria are driven by proton or sodium motive force, motility can be viewed simply as a special case of ion turnover. MacNab and Koshland (58) indicated that as much as 1% of the total energy in *E. coli* could be devoted to motility. Because the maintenance rate of *E. coli* is only 1.25% of the glucose consumption rate of exponentially growing cells (85), this value is probably not a precise estimate.

## $Y_{ATP/MAX}$

In an effort to account for the impact of maintenance on $Y_{ATP}$, Stouthamer and Bettenhaussen (108) introduced a new term, $Y_{ATP/MAX}$, which was corrected for maintenance energy. However, DeVries et al. (26) noted that not even this correction could give $Y_{ATP}$ values as great as 32 g of cells per mol; *Lactobacillus casei* grown in glucose-limited continuous cultures had a $Y_{ATP/MAX}$ of only 24.3 g of cells per mol of ATP. Because other values of $Y_{ATP/MAX}$ were even lower, it appeared that maintenance energy alone could not explain the variations in growth efficiency (Table 3). As noted by Harold (42), "there is something misleading about the fundamental assumption that the free energy of catabolism is fully conserved as ATP and expended necessarily either for biosynthesis or for useful work. Any departure from perfect coupling, either in the generation of ATP or in its utilization, will show up as a shortfall of the yield and exaggerate the apparent cost of cellular upkeep."

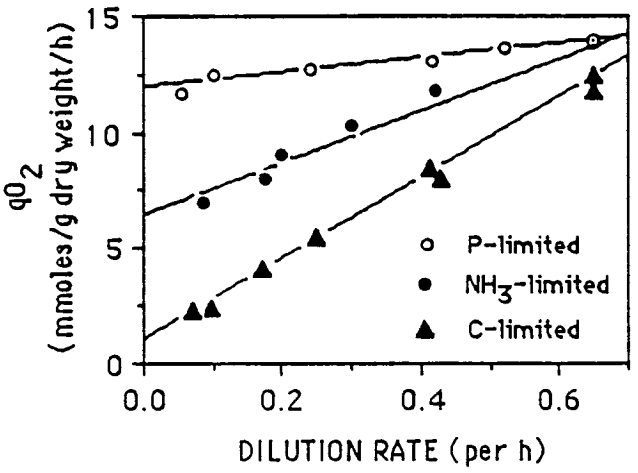


FIG. 4. Relationship between the specific growth rate and the specific rate of $O_2$ consumption in chemostat cultures of *Klebsiella aerogenes* growing in glucose-containing media that were limited by carbon, phosphorus, or ammonia. Reprinted with permission from reference 117.

## IS MAINTENANCE ENERGY A CONSTANT?

Hempfling and Mainzer (44) grew *E. coli* in continuous culture and noted that the specific rate of oxygen consumption was dependent on the carbon source limiting growth as well as on the growth rate of the cultures. Even when corrections were made for differences in ATP production, the maintenance coefficients varied by as much as 2.5-fold. Anderson and von Meyenburg (1) likewise observed that the specific rate of respiration by *E. coli* was not well correlated with the growth rate when the carbon source was changed. Since the variation in respiration was far greater than the amount that could be ascribed to ATP production, it appeared that the cells had a variable maintenance coefficient or were wasting (spilling) ATP.

When Neijssel and Tempest (74, 75) grew *Klebsiella aerogenes* in continuous cultures which were limited by carbon, ammonia, sulfate, or phosphate, the rate of carbon source utilization was always higher when carbon was in excess. On the basis of Tempest plots (Fig. 4), "these carbon-sufficient cultures had a greatly increased maintenance energy requirement, but nevertheless used the remaining energy with a much increased efficiency compared with carbon-limited cultures." Maintenance (the intercept of the Tempest plot) increased but the slope ($1/Y_G$) decreased when carbon was in excess.

Because mannitol- and glucose-limited cultures of *Klebsiella aerogenes* had significantly lower growth yields than did gluconate-limited cultures, Neijssel and Tempest (76) indicated that "the mannitol and glucose-limitations must be essentially

carbon (and not energy) limitations." In an effort to explain the difference between "energy- and carbon-limited" cultures, Neijssel and Tempest concluded that "maintenance energy is composed of at least two factors: (i) maintenance of cell integrity, and (ii) maintenance of growth potential (involving slips reactions)." On the basis of these latter assumptions, an additional variable, $c$, was added to the standard Pirt equation (75):

$$\frac{1}{Y} = \frac{m}{\mu} + \frac{1}{Y_G} + c \cdot m$$

Since $q = (1/Y)\mu$ and $1/Y = q/\mu$,

$$\frac{q}{\mu} = \frac{m}{\mu} + \frac{1}{Y_G} + c \cdot m$$

Multiplying by $\mu$,

$$q = m + \mu[(1/Y_G) + c \cdot m]$$

In this model of energy excess cultures, the $c$ term is given a negative value. The negative $c$ term allows for a decrease in $1/Y_G$, the slope, but this adjustment alone would not account for an increase in $m$, the intercept (Fig. 4).

Pirt (86) addressed the idea of variable maintenance rate by redefining maintenance with both growth rate-independent ($m$) and growth rate-dependent ($m'$) components in which equation 1 is modified. $k\mu$ is defined as the specific growth rate-dependent maintenance rate:

$$\frac{1}{Y} = \frac{m}{\mu} + \frac{1}{Y_G} + \frac{m'(1\text{-}k\mu)}{\mu}$$

Since $q = (1/Y)\mu$ and $1/Y = q/\mu$,

$$\frac{q}{\mu} = \frac{m}{\mu} + \frac{1}{Y_G} + \frac{m'(1-k\mu)}{\mu}$$

Multiplying by $\mu$,

$$q = m + \mu(1/Y_G) + m'(1 - k\mu)$$

$k$ is assigned a value of 1 when the culture is growing at its maximum rate or is carbon limited, but $k$ increases to values greater than 1 when carbon is in excess. This unified model provided a realistic method of describing the variation in $m$ but did not address the biological mechanisms affecting the variable maintenance. As Pirt noted (86), "Why should the specific maintenance rate ($a$) vary up to 30 fold (from about 0.01 to 0.3 $h^{-1}$) depending on the nature of the carbon and energy source which limits growth?" In his review of factors affecting the growth rate of *E. coli*, Marr (62) indicated that there is a "considerable body of evidence" indicating that ATP production does not necessarily determine the growth rate of *E. coli*, even if all necessary nutrients are in excess. Recent work with *Zymomonas mobilis* indicated that overexpression of fermentative genes in some cases caused a 50% reduction in glycolytic rate, but the rate of growth did not decrease (2).

## OTHER MECHANISMS OF ENERGY LOSS

The terms "uncoupling," "energy spilling," "overflow metabolism," "futile cycles," "slip reactions," and "wastage" (11, 62, 76, 102, 107, 116) have all been used as corrective measures to justify variations in yield, but the mechanism(s) of the additional energy expenditure was not defined. In the 1980s, Westerhoff et al. (129–131) applied the principles of nonequilibrium thermodynamics to the study of bacterial growth and

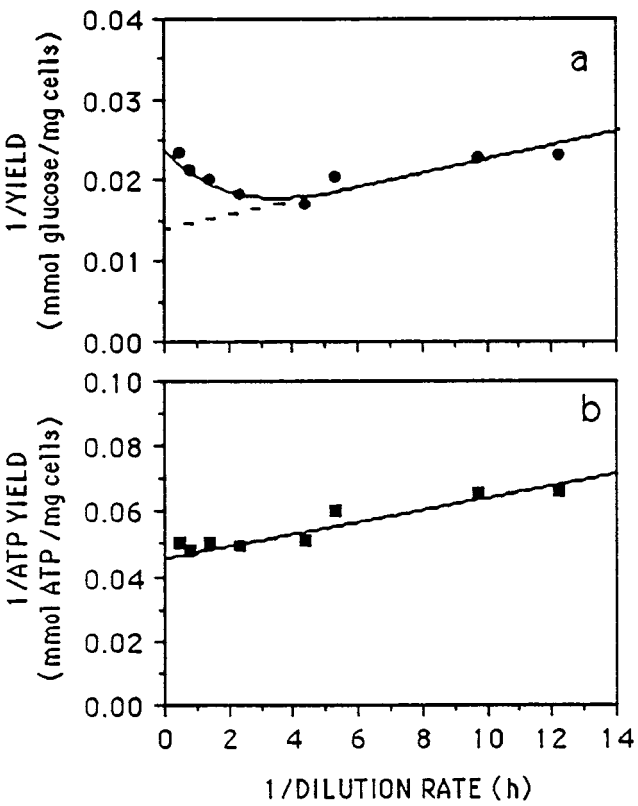


FIG. 5. Effect of dilution rate on the glucose yield (a) and ATP yield (b) of *Streptococcus bovis* when it was grown in continuous culture. Redrawn from the data of Russell and Baldwin (97).

concluded that microbial growth yields were "50% less than they theoretically could be" and that "anabolism is incompletely coupled to catabolism." According to Westerhoff et al. (129), "some thermodynamic efficiency may be sacrificed to make the process run faster," but the carbon-sufficient cultures of Neijssel and Tempest (75) had their greatest unexplained energy dissipation when the growth rate was low (Fig. 4).

### Overflow Metabolism

Bacteria sometimes excrete or leak partially oxidized metabolic intermediates, capsular material, and protein into culture media (21, 38, 101, 107, 119). Tempest and Neijssel (74, 117) noted that *Klebsiella aerogenes* produced pyruvate, 2-oxoglutarate, gluconate, 2-ketogluconate, and succinate when energy was in excess, but these intermediates accounted for less than 50% of the unexplained energy source utilization (75). The cells also excreted some extracellular polysaccharide and protein, but even these products could not account for the abnormally high rates of glucose consumption (74). Since the carbon balances were nearly 100%, it appeared that the cells were, indeed, respiring the glucose and not just secreting or storing carbon.

### Metabolic Shifts

Fermentative bacteria can change their end products and alter ATP production. Many streptococci and lactobacilli that were originally classified as homolactic produce acetate, formate, and ethanol when the rate of glucose fermentation is low (26, 97). The change from a homo- to heterofermentative scheme increases the ATP/glucose ratio from 2 to 3. These

changes are regulated by fructose 1,6-diphosphate (FDP), an allosteric effector of lactate dehydrogenase (LDH) (132). When the rate of glucose fermentation decreases, the level of intracellular FDP declines and the LDH is no longer activated.

In *Streptococcus bovis*, the FDP activation is also regulated by changes in intracellular pH. As intracellular pH declines, the LDH requires less FDP and the fermentation is homolactic even though the rate of glucose fermentation is low (98). *Selenomonas ruminantium* also regulates lactate production as a function of fermentation rate (97), but this regulation is not mediated by the effect of FDP on the LDH (126). Wallace (126) indicated that lactate regulation by *S. ruminantium* might be mediated by a homotropic activation of the LDH by pyruvate, but later work indicated that the intracellular pyruvate concentration did not increase (112). In *S. ruminantium*, the decrease in lactate production at low rates of glucose fermentation is associated with an increase in levels of acetate, propionate, and sometimes succinate (97, 112).

When *S. bovis* was grown in a glucose-limited chemostat, the glucose yield declined at high dilution rates, and this decrease was caused by a change from hetero- to homolactic fermentation (97). The plot of (1/dilution rate) versus (1/glucose yield) was linear at low dilution rates when virtually all of the glucose was converted to acetate, formate, and ethanol, but 1/yield increased at high dilution rates (Fig. 5a). When corrections were made for differences in ATP production, the plot was linear (Fig. 5b). On the basis of the intercept of the plot, the $Y_{ATP/MAX}$ was 22 g of cells per mol of ATP. Even when corrections were made for the incorporation of glucose into cell carbon, the $Y_{ATP/MAX}$ was only 25 g of cells per mol of ATP (93).

In the 1930s, methylglyoxal was believed to be a normal intermediate in the catabolism of glucose, but by the 1940s, the methylglyoxal shunt was largely dismissed as an artifact (19). In 1970, Cooper and Anderson (20) showed that *E. coli* used a pathway involving methylglyoxal to convert dihydroxyacetone phosphate to D-lactate. Since this pathway does not have phosphate transferases, the free energy change of glucose catabolism does not generate ATP. The methylglyoxal pathway has been demonstrated in *Pseudomonas saccharophilia*, *Clostridium sphenoides*, and *Enterobacter* (*Klebsiella*) *aerogenes* (19, 34, 35).

When anaerobic glucose-limited continuous cultures of *K. aerogenes* were pulsed with glucose, the specific rate of glucose consumption increased markedly, and much of the additional glucose was converted to D-lactate (114, 115). From this and subsequent work (110, 111), it appeared that anaerobic cultures of *K. aerogenes* could shift their metabolism and produce less ATP when an energy source was in excess. However, not even the methylglyoxal shunt could explain all of the nongrowth, nonmaintenance energy dissipation of *K. aerogenes*. When *K. aerogenes* was grown aerobically with an excess of glucose under steady-state conditions, the specific rate of oxygen consumption was greater than under glucose-limited conditions and D-lactate could not be detected as an end product (74, 75).

In some cases, metabolic shifts account for some of the variations in yield, but such a phenomenon alone cannot explain why the $Y_{ATP/MAX}$ of most bacteria is significantly lower than the theoretical value of 32 g of cells per mol of ATP that was derived by Stouthamer (106).

### Uncoupling

Senez (102), in describing the link between energy-yielding reactions and the energy-consuming reactions of cell biosynthesis, conceptualized any anomaly as "uncoupling." This all-inclusive definition did not differentiate between the production of ATP and the utilization of ATP in nongrowth reactions. Because the latter process would be more aptly termed ATP spilling (see the section on ATP spilling, below), we will define uncoupling as the inability of chemiosmotic mechanisms to generate the theoretical amount of metabolic energy.

Oxygen consumption is often used as an indicator of respiration, but as Haddock (39) noted, "it is important to appreciate that not all membrane-bound redox enzymes synthesized in *E. coli* are necessarily involved in energy conservation. Many serve simply for the reoxidation of reduced coenzymes, the removal of potentially toxic metabolic products, or the reduction of intermediates required for biosynthetic reactions." *Azotobacter vinelandii* uses a portion of its respiratory chain solely to scavenge oxygen and protect nitrogenase (51), and when *E. coli* was grown under sulfate limitation, the NADH dehydrogenase became a non-proton-translocating enzyme (87). *E. coli* has two different respiratory pathways involving different NADH dehydrogenases (NDH-1 and NDH-2) and terminal cytochromes (*o* and *d*) (40). An *E. coli* mutant defective in cytochrome *o* grew less efficiently than the wild type, but a mutant defective in NDH-2 grew with greater efficiently than the wild type (15). From these comparisons, it appears that bacteria can have multiple strategies of electron flow and coupling.

Because ATPase-negative mutants can use electron transport systems to create a $\Delta p$ but not ATP, ATPase-negative mutants have been used as a method of estimating coupling. Jensen and Michelsen (49) recently reported that ATPase-negative mutants of *E. coli* had much higher rates of oxygen consumption than could be explained by a simple shift from oxidative phosphorylation to glycolysis and concluded that wild-type *E. coli* was not coupling respiration and ATP synthesis in a highly efficient manner (49). On the basis of the inherent constraints of estimating the degree of coupling that exists in oxidative phosphorylation, one must view the $Y_{ATP}$ values of aerobes with a high degree of skepticism. Only in anaerobic systems which depend solely on substrate level phosphorylation can the rate of ATP production be estimated with any certainty.

Stouthamer and Bettenhaussen (109) noted that an ATPase mutant grown aerobically had a $Y_{ATP/MAX}$ that was more than twice as high as that of a wild type grown anaerobically and concluded that wild-type *E. coli* was using more than half of its energy to sustain a membrane potential. Since the turnover of ions through the cell membrane is clearly a maintenance function, one would have expected a difference in the *m* coefficient, but there was little difference in either $m_{glucose}$ or $m_{ATP}$. 2,4-Dinitrophenol, an uncoupler which acts as a protonophore, caused a decrease in theoretical maximum growth as well as an increase in maintenance energy (73).

## ENERGY-SPILLING REACTIONS

### Futile Cycles

**Futile enzyme cycles.** Certain sequences of metabolism can serve as catabolic and anabolic pathways (e.g., glycolysis and gluconeogenesis) and act in an antagonistic fashion (e.g., phosphofructokinase and fructose-1,6-diphosphatase, glycogen synthetase and glycogen glycogenolysis, glucokinase or the glucose phosphotransferase system (PTS) and glucose-6-phosphatase) (Fig. 6a). These antagonistic enzymes must be regulated to prevent a futile cycle of ATP utilization. Bacteria have evolved a variety of allosteric mechanisms to counteract these opposing

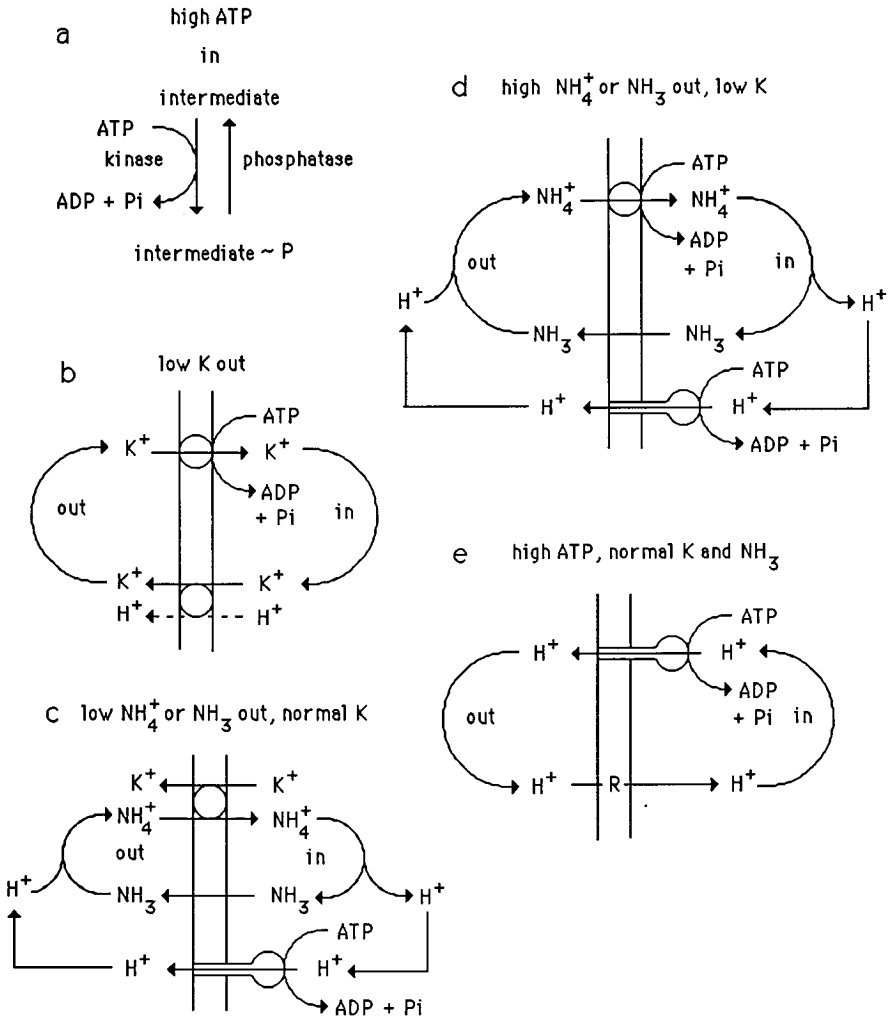


FIG. 6. Potential methods of energy spilling in bacteria. (a) Futile cycles of enzymes involving phosphorylation and dephosphorylation. (b) A futile cycle of $K^+$ via $K^+$ influx by high-affinity $K^+$ transport and $K^+$ efflux via low-affinity $K^+$ transport. (c) Uptake of $NH_4^+$ via $K^+/NH_4^+$ antiporter, dissociation of $NH_4^+$, passive efflux of $NH_3$, and efflux of $H^+$ via the $F_1F_0$-ATPase. (d) Uptake of $NH_4^+$ by high-affinity $K^+$ transport, dissociation of $NH_4^+$, the passive efflux of $NH_3$, and expulsion of $H^+$ via the $F_1F_0$-ATPase. (e) Expulsion of $H^+$ via the $F_1F_0$-ATPase and the influx of $H^+$ via a resistance (R) change in the cell membrane.

processes, and these mechanisms can respond to key intracellular metabolites as well as to the energy state of the cell.

Otto (81) reported that *Lactococcus* (*Streptococcus*) *cremoris* had both phosphofructokinase and fructose-1,6-diphosphatase activity and suggested that these two enzymes were responsible for the increased rate of lactose catabolism by leucine-limited cells. This conclusion was based on the observation that the leucine-limited cells had fivefold-lower intracellular AMP levels and less phosphoenolpyruvate than did the lactose-limited cells. AMP is an inhibitor of fructose-1,6-diphosphatase, and phosphoenolpyruvate is an inhibitor of phosphofructokinase. Direct flux through this cycle, however, was not demonstrated. Using $^{32}P_i$ labeling, Daldal and Fraenkel (22) noted that there was little, if any, gluconeogenic futile cycling in exponentially growing *E. coli* cells.

Patnaik et al. (83) recently examined the potential cycle of pyruvate kinase and phosphoenolpyruvate synthase in *E. coli* by using overexpression mutants. The mutants consumed more oxygen than did the wild type, but a 30-fold overexpression of phosphoenolpyruvate synthase increased oxygen consumption only twofold. When Chao and Liao (16, 17) overexpressed phosphoenolpyruvate carboxylase in *E. coli*, the cell yield increased, but most of this effect was explained by an increase in ATP production (less fermentation) rather than less ATP turnover per se. From these results, it did not appear that phosphoenolpyruvate carboxylase and phosphoenolpyruvate carboxykinase were operating as a significant futile cycle.

2-Deoxyglucose (2-DG) is often used to deenergize bacteria. 2-DG is taken up by the glucose PTS, 2-DG-6-phosphate is accumulated to high concentrations (approximately 100 mM), 2-DG-6-phosphate is dephosphorylated by hexose-6-phosphatase, and 2-DG leaks out of the cell (121). Since the phosphoenolpyruvate must be replenished, this cycle represents a net loss of ATP. The potential involvement of sugar uptake and efflux in bacterial growth kinetics has never been assessed, but it is unlikely that glucose PTS and hexose-6-phosphatase would operate as a futile cycle under physiological conditions. The intracellular concentration of glucose 6-phosphate in glycolyzing (most probably energy-spilling) cells was only 1.6 mM (121), the affinity of the phosphatase for hexose 6-phosphate was very low ($K_m$ of approximately 20 mM) (122), and the

TABLE 4. Effect of futile enzyme cycles on the ATP turnover rate by bacteria under different growth conditions

| Bacterium | Growth limitation | Nongrowth energy dissipation | Additional ATP consumption (μmol of ATP/mg of protein/h) | Reference |
|---|---|---|---|---|
| *E. coli* | Glucose | Maintenance | 5.1–8.7 | 109 |
| *E. coli* | Potassium | Energy spilling | 19.2 | 70 |
| *E. coli* | Potassium + excess ammonium | Energy spilling | 25.4 | 13 |
| *S. bovis* | Glucose | Maintenance | 5.0 | 97 |
| *S. bovis* | Chloramphenicol | Energy spilling | 50.8 | 18 |

addition of glucose broke the cycle of deenergization by 2-DG (121).

*E. coli* carefully regulates its rates of glycogen synthesis and glycogenolysis (88), but such control may not be a ubiquitous feature of all bacteria. When the glycogen reserves of the cellulolytic bacterium *Fibrobacter succinogenes* were labeled with [$^{13}$C]glucose and the cells were then incubated with [1-$^{13}$C]glucose and [2-$^{14}$C]glucose, there was a rapid loss of both 1-$^{13}$C and 2-$^{13}$C (36). From these results, the authors concluded that "glycogen was degraded at the same time as it was being stored, suggesting futile cycling of glycogen." Subsequent work, however, indicated that glycogen recycling in *F. succinogenes* was not a primary component of its maintenance energy. The specific rate of glycogen catabolism in *F. succinogenes* was always at least threefold lower than the maintenance rate of growing cells (127).

While it is impossible to rule out futile enzyme cycles as a potential mechanism of ATP turnover in bacteria, there is little evidence to suggest that such cycles are highly significant or likely to play a major role in ATP spilling.

**Futile cycles of potassium and ammonium.** In *E. coli*, intracellular potassium is a prime factor in regulating turgor pressure, and this bacterium has multiple transport systems which are involved in potassium uptake and efflux (3). Similar systems appear to operate in a variety of gram-negative bacteria and *Staphylococcus aureus* (27). Mulder et al. (70) studied the impact of potassium transport systems on the growth efficiency of *E. coli* when potassium was limiting. Because a mutant which was defective in high-affinity potassium transport utilized glucose more efficiently, it appeared that the wild type was taking up potassium by the high-affinity, ATP-driven (Kdp) system and losing potassium through the low-affinity proton symport (Trk) system (Fig. 6b). On the basis of differences in ATP production rates and the steady-state concentrations of biomass in continuous culture, it appeared that the futile cycle of potassium was decreasing ATP availability (Table 4).

The uptake and efflux of potassium proposed by Mulder et al. (70), however, would not necessarily lead to a futile energy cycle (Fig. 6b). The uptake of potassium via the ATP-driven Kdp system would consume ATP, but electrogenic efflux of potassium via the Trk potassium proton symport would generate a $\Delta\Psi$ and decrease $F_1F_0$-ATPase-dependent ATP hydrolysis. A significant energy loss would only occur if there was another pathway of potassium efflux (e.g., a potassium channel operating as a uniporter). Zoratti and Ghazi (134) recently summarized evidence for the existence of turgor-activated potassium channels in *E. coli*. These potassium uniporters seem to provide a more plausible explanation for the results of Mulder et al. (70) than does the Trk per se.

When ammonia concentrations are high, facilitated diffusion appears to be the dominant mechanism of ammonia uptake, but bacteria also have active uptake mechanisms for ammonium ions (4, 52). Since internal $K^+$ is required for the accumulation of [$^{14}$C]methylammonium and ammonium competes with methylammonium uptake, it appears that *E. coli* has a $K^+/NH_4^+$ antiporter (4). Active uptake of ammonium ion, however, is counteracted by a more alkaline interior and the passive efflux of ammonia. Kleiner (52) estimated that up to 6 mol of ammonium may be transported before 1 mol can be fixed by the glutamine synthetase/glutamate synthase cycle (Fig. 6c). This futile cycle of ammonium and ammonia would decrease $\Delta p$, dissipate the potassium gradient, and increase $F_1F_0$-ATPase activity.

When potassium is limiting, some bacteria can use ammonium as a replacement ion for intracellular potassium (13), and under these conditions *E. coli* appears to transport ammonium ions via the Kdp potassium uptake system (12). When *E. coli* was grown under potassium-limiting conditions, addition of ammonium chloride caused a significant increase in the specific rate of oxygen consumption (12). On the basis of the observation that a mutant which lacked the Kdp potassium transport system grew more efficiently under potassium limitation when ammonium was in excess, it appeared that ammonium was being taken up by Kdp and was then diffusing out of the cell as ammonia (Fig. 6d). In this case, the futile cycle would result in a direct consumption of ATP by Kdp as well as increased ATP consumption by the $F_1F_0$-ATPase.

**Futile cycle of protons.** The fermentative bacterium *Streptococcus bovis* derives all of it energy from substrate-level phosphorylation, and continuous-culture studies indicated that this bacterium had a high $Y_{ATP/MAX}$ (30 g of cells per mol of ATP) if it was glucose limited (93). When *S. bovis* was grown in batch culture with an excess of glucose, the $Y_{ATP/MAX}$ declined more than 15%, and chloramphenicol-treated batch cultures fermented glucose at a high rate even though growth and protein synthesis were completely inhibited (18, 93). This nongrowth glucose consumption rate was 10 times the maintenance rate of glucose-limited cells and nearly one-third the rate of exponentially growing cells (approximately 28 μmol of glucose per mg of protein per h).

Because *S. bovis* spilled energy even when potassium and ammonium were in excess (18), it appeared that the cells had a mechanism of energy spilling that did not involve high-affinity potassium or ammonium carriers (Fig. 6c and d). This hypothesis was supported by the observation that *N*,*N*′-dicyclohexylcarbodiimide (DCCD), an inhibitor of the membrane-bound $F_1F_0$-ATPase, completely inhibited nongrowth glucose fermentation and ATP turnover. Thus, the ATP spilling by *S. bovis* seemed to be caused by a direct cycle of protons through the cell membrane (Fig. 6e). This model was consistent with the effect of 3,3′,4′,5-tetrachlorosalicylanilide (TCS), an uncoupler that decreases membrane resistance to protons. When the chloramphenicol-treated *S. bovis* cells were treated with TCS, the rate of energy spilling increased more than twofold (100). The idea that bacteria can decrease their membrane resistance is supported by the work of Taylor and Jackson (113), who showed that the phototrophic bacterium,

*Rhodobacter capsulatus*, had a current-carrying pathway in the cell membrane that was capable of dissipating a light-driven membrane potential when energy was in excess. Because only net charge transfer was measured, the current could not be precisely defined, but proton flux was "the most likely candidate."

The best-documented example of a proton cycle occurs in the mitochondria of brown adipose tissue, and this cycle is mediated by a specific proton channel. For many years, it was assumed that energy spilling was a unique characteristic of newborn and hibernating animals, but recent work indicated that mitochondrial proton leak is a general characteristic of most mammalian tissues (7). Brand et al. (7) estimated that proton leaks could account for 26% of the total oxygen consumption of animals. The mechanism of the proton leak in tissues like muscle and liver has been linked only to long term effects (hormone actions, increased surface area of mitochondrial membranes, and changes in the fatty acid composition of mitochondrial membranes).

**Comparison of futile ion cycles.** The experiments with *E. coli*, *S. bovis*, and *R. capsulatus* indicate that bacteria can dissipate energy in futile cycles of ions through the cell membrane and that these cycles can be regulated by short-term mechanisms that do not involve additional protein synthesis. Some ion cycles will occur only under specific nutrient limitations (e.g., potassium and ammonia), but proton cycles can operate whenever there is an imbalance of catabolic and anabolic rates. This additional nongrowth energy dissipation will detract from cell production in the same manner as maintenance, but the magnitude is often much greater. In *S. bovis*, the rate of energy spilling is 10-fold greater than the maintenance coefficient and approximately one-third of the glucose consumption rate of exponentially growing cells. Given such observations, energy spilling could have a very significant effect on the overall efficiency of bacterial cell production and explain much of the discrepancy between actual and theoretical growth yields (Table 4).

### Are Energy-Spilling Reactions Constitutive or Inducible?

Because ATP-spilling reactions were traditionally demonstrated in continuous cultures that were limited by nutrients other than energy, it appeared that ATP spilling might be an inducible phenomenon. This assumption was supported by the observation that high-affinity transport systems for ammonium and potassium are clearly inducible (Fig. 6b to d). Pulse dose experiments, however, indicated that bacteria in even rich media could spill excess energy. When energy-limited continuous cultures of *Pseudomonas* sp. (9), *K. aerogenes* (76), *Selenomonas ruminantium*, *Prevotella ruminicola* (92), and *Streptococcus bovis* (18) were given a pulse dose of energy source, there was an immediate increase in the rate of energy source utilization that did not correspond to an increase in cell production (energy spilling). Because *S. bovis* showed an immediate increase in energy spilling when it was treated with chloramphenicol (18, 100) and energy-limited *Rhodobacter capsulatus* had the same inherent capacity to spill energy as energy-excess cultures (113), it does not appear that energy spilling is an adaptive phenomenon that requires additional protein synthesis.

It has long been recognized that many bacteria grow faster and more efficiently when amino acids are present in the growth medium (24, 30, 31, 48), but Stouthamer's calculations indicated that amino acids should have very little impact on the efficiency of biomass production (Table 1). When *S. bovis* was grown in glucose-limited continuous cultures, amino acids had no effect on either $Y_G$ or the $m$ coefficient; however, amino acids caused large increases in the yield and growth rate of batch cultures. Because the amino acid-dependent change in growth rate and yield of the batch cultures was at least five times greater than the maintenance rate of glucose-limited cells, maintenance alone could not explain the difference in growth efficiency.

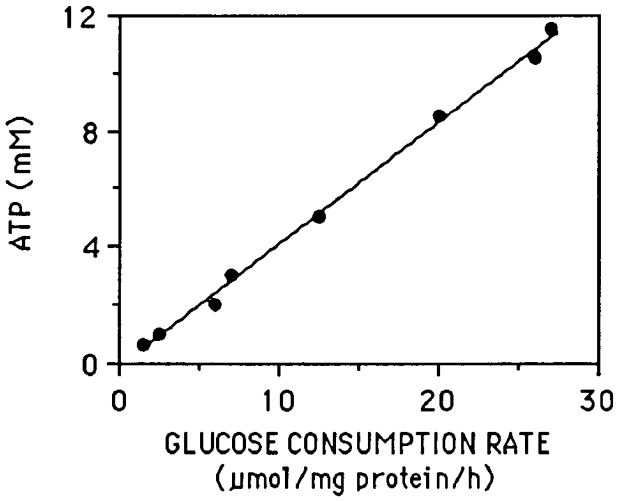


FIG. 7. Relationship between the rate of glucose consumption and intracellular ATP concentration in nongrowing, energy-spilling *Streptococcus bovis* cells. Redrawn from the data of Cook and Russell (18).

The effect of amino acids on the growth of *S. bovis* is most easily explained by energy spilling and the balance of anabolic and catabolic rates (95). In continuous cultures, the rate of anabolism was regulated by the rate of glucose entry (dilution rate), the cells were energy limited, and the efficiency of growth was high. In the batch cultures, the situation was quite different. Glucose was always in excess, and the rate of anabolism was controlled by amino acid availability. When *S. bovis* was transferred from a rich medium (containing amino acids) to a minimal medium (only ammonia as a nitrogen source), the specific growth rate decreased by 50% but the specific rate of glucose consumption remained the same (95). Since the catabolic and anabolic rates were no longer in balance, the additional ATP was hydrolyzed by energy-spilling reactions.

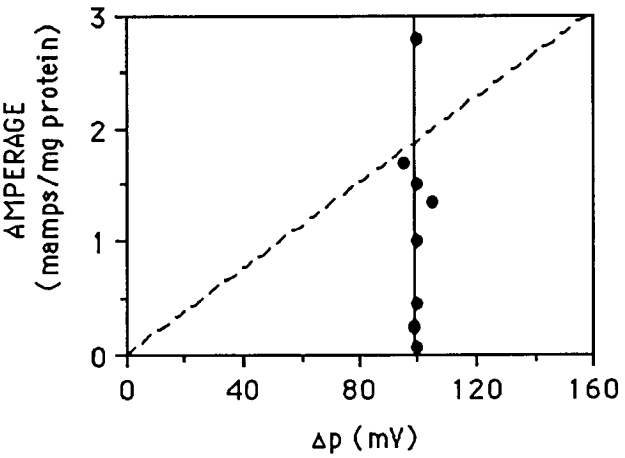


FIG. 8. Relationship between $\Delta p$ and amperage in nongrowing, energy-spilling *Streptococcus bovis* cells. Reprinted with permission from reference 18. The broken line represents a hypothetical Ohmic relationship.

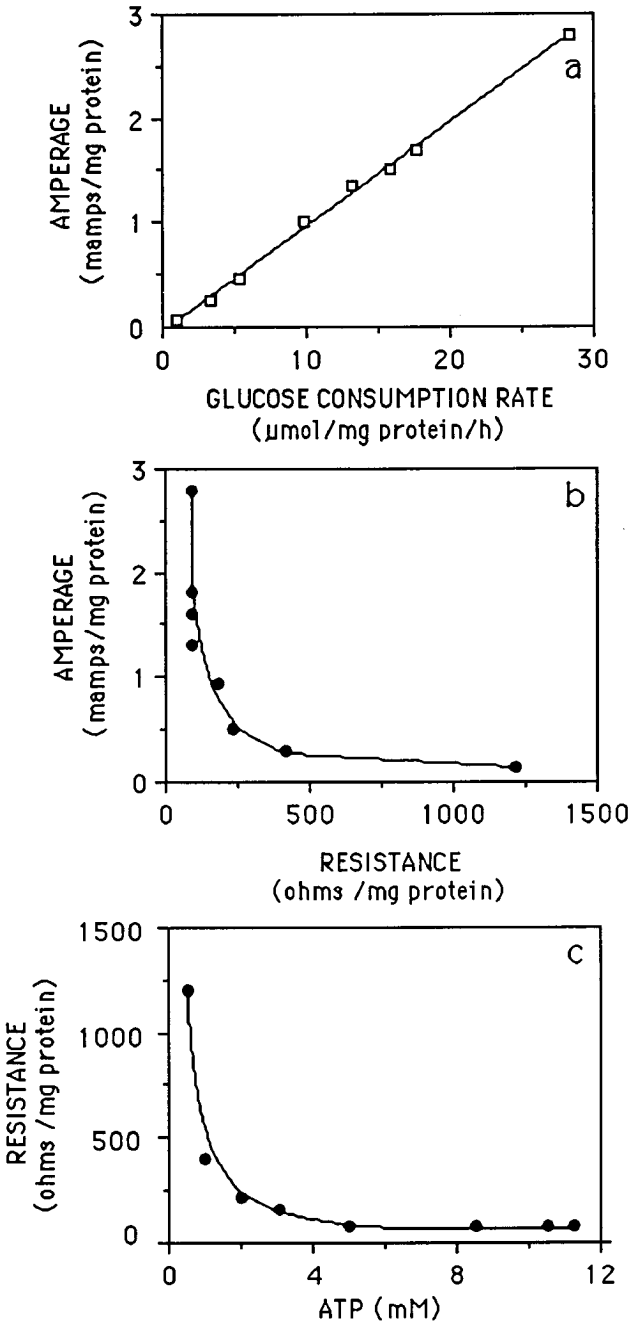


FIG. 9. Relationships between the rate of glucose consumption (energy spilling) and amperage (a), membrane resistance and amperage (b), and ATP versus membrane resistance (c) of nongrowing, energy-spilling *Streptococcus bovis* cells. The rate of energy spilling was regulated by the addition of iodoacetate. Reprinted with permission from reference 18.

In his review, Marr (62) indicated that there was little evidence that the flux of ATP ever controlled the maximum growth rate of *E. coli* and speculated that maximum growth rate was usually set by "the flux of a precursor metabolite." This observation is consistent with the supposition that energy-spilling reactions are (i) a common feature of growth with an excess of energy, and (ii) an indicator of the imbalance between anabolism and catabolism.

### How Are Energy-Spilling Reactions Regulated?

Because energy-limited cultures do not normally spill ATP, bacteria must have a mechanism of assessing energy status. *Streptococcus bovis* did not spill energy until glucose accumulated (18), but the extracellular glucose concentration alone could not explain the effect of nitrogen source (ammonia versus amino acids) on the energy-spilling rate of *S. bovis* (95). When chloramphenicol- or nitrogen-limited *S. bovis* cultures were treated with iodoacetate, the rate of nongrowth glucose consumption was directly proportional to the intracellular ATP concentration (Fig. 7) (18), and this result indicated that ATP might be a regulator of energy spilling.

Lazdunski and Belaich (54) hypothesized that *Zymomonas mobilis* had two ATPase activities, a high-affinity system that generated a proton pump and a low-affinity type that functioned only as an ATPase. Their studies, however, involved only cell extracts and did not correlate ATPase activity with $\Delta p$ formation. When energy-spilling *S. bovis* cells were treated with the protonophore TCS, the rate of glucose consumption and ATP turnover increased threefold (100). This result indicated that the ATPase activity was being regulated, either directly or indirectly by $\Delta p$, but later work indicated that the magnitude of $\Delta p$ did not change significantly (Fig. 8).

Proton flux is usually estimated from the rate of ATP production and the $\Delta G$ of ATP hydrolysis (53), but there is little proof that the ATPase can harvest all of the $\Delta G$ to pump protons (18). Microcalorimetry allows for a more direct estimate of proton flux and is based on the simple assumption that the amperage (proton flux) of an electrical circuit must be equal to the heat production (watts) divided by the voltage. Since heat is an estimate of $\Delta H$, such measurements are not confounded by the problems of $\Delta G$ estimation. Some of the heat is also produced by nonmembrane-linked reactions (e.g., glycolysis), but in nongrowing *S. bovis* cells the membrane heat was a simple function of ATP production and the $\Delta H$ of ATP (18).

$\Delta H$ measurements indicated that the rate of proton flux in *S. bovis* was, indeed, a direct function of the glucose consumption rate (Fig. 9a), and Ohm's law, in turn, indicated that the resistance of the cell membrane to proton conductance was changing (Fig. 9b). When the rate of energy spilling was high, the resistance decreased, the rate of proton entry increased, and more ATP was expended to pump the protons back out. Because the decrease in membrane resistance was observed only when the ATP level was high (Fig. 9c), it once again appeared that intracellular ATP might be the regulator of the futile cycle of protons.

In *S. bovis*, $\Delta p$ is generated by the $F_1F_0$-ATPase, but the phototroph *Rhodobacter capsulatus* generates $\Delta p$ by a light-driven mechanism that does not require ATP. When Taylor and Jackson (113) treated *R. capsulatus* with the ATPase inhibitor venturicidin, the cells had virtually no ATP, but membrane resistance changes could still be observed (113). Taylor and Jackson (113) hypothesized that the membrane resistance changes of *R. capsulatus* might be caused by a change in the membrane ATPase. In their scheme, the $\Delta p$ threshold of the ATPase would be close to the physiological $\Delta p$ when energy is limiting, most of the $\Delta p$ would be used to drive ATP formation, and growth would be efficient. In cases when anabolism is restricted and energy is in excess, the threshold of the ATPase for $\Delta p$ would be shifted to a lower value, the excess $\Delta p$ would be used to drive a futile cycle of protons, and growth efficiency would decrease. This model, however, introduces yet another question. Why would the ATPase threshold change?

In mammals and lower animals, the resistance of the cyto-

plasmic membrane seems to be regulated in a relatively straightforward manner. When the rate of catabolism is high, cytoplasmic pH drops, and this change in pH causes voltage-gated channels to open so that excess protons can be eliminated (24a). Mammalian mitochondria also seem to regulate their membrane resistance, but the pathway of proton conductance and its regulation have not been so precisely defined. The possibility of voltage-regulated channels in bacteria is supported by the effect of glucose on *S. bovis*. When glucose-limited cells were given a pulse dose of glucose, there was a rapid shift from energy conservation to energy spilling, and this shift in energetics was correlated with a transient increase in membrane voltage (18). Further work is clearly needed to define the regulation of proton conductance in bacterial membranes.

### Is Energy Spilling Advantageous?

**Bacterial competition.** In natural environments, bacteria often compete intensely for the same energy source, but energy is not the only factor that can limit bacterial growth. When a bacterium is limited by factors other than energy, energy spilling could be advantageous. If energy that would otherwise go to a potential competitor could be wasted, a bacterium could conceivably position itself in a more favorable future situation. Such strategies are often used by humans, but there is little evidence that bacteria are able to predict the future and scheme for future success.

A less teleological argument for energy spilling might be the rapid reinitiation of growth. As noted by Neijssel and Tempest (76), energy spilling has the potential of allowing bacteria to "accelerate their processes of cell synthesis without being impeded by a lack of available energy (ATP)." Concomitantly, the continued high rate of energy source turnover might also provide higher intracellular concentrations of critical precursor metabolites (62). Either of these strategies would have direct and immediate selective value.

**Dielectric effects.** When polar substances (e.g., phospholipids) are exposed to an electric field, the molecules will act as dielectrics and be realigned by the charge distribution. Cell membranes have a high capacitance and can theoretically sustain an electrical potential of approximately 500 mV (42), but such estimates do not consider dielectric effects. The $\Delta p$ of bacteria is generally threefold lower than the $\Delta G'p$ of ATP hydrolysis (18), but, as mentioned above (see the section on factors affecting $Y_{ATP}$ determination), direct estimates of amperage indicated that the proton stoichiometry ($n$) of the $F_1F_0$-ATPase was only 1.9 (6, 18, 84). When $n$ is low, however, $\Delta p$ could theoretically be very high:

$$\Delta p = \Delta G'p/n$$

A decrease in membrane resistance offers a bacterium a means of decreasing $\Delta p$ (voltage $\alpha$ resistance) and protecting its cell membrane from potentially deleterious dielectric effects that could distort membrane structure. DeCoursey and Cherny (24a) recently speculated that proton channels may serve as "a safety valve" in situations of excessive metabolic activity.

**Methylglyoxal toxicity.** The ruminal bacterium *Prevotella ruminicola* showed only a small increase in the specific rate of glucose consumption when it was shifted from glucose to nitrogen limitation in continuous culture, and most of the additional glucose was stored as polysaccharide (94). *P. ruminicola* had little capacity to spill energy, but these nitrogen-limited cultures were highly unstable (94). The nitrogen-limited continuous cultures washed out of continuous culture, even though the dilution rate was low, and the viability of nitrogen-limited batch cultures decreased by more than 10,000-fold. Subsequent work indicated that the cell death was caused by methylglyoxal accumulation (96). The nitrogen-limited cultures produced as much as 3 mM methylglyoxal, and *E. coli* can be completely inhibited by as little as 0.25 mM (123). Methylglyoxal was initially categorized as an inhibitor of DNA replication and protein synthesis (34), but it can also have short-term effects. In *P. ruminicola*, methylglyoxal caused a rapid decline in $\Delta\Psi$ and intracellular potassium concentration (96).

In *E. coli*, methylglyoxal production is observed when xylose, glucose 6-phosphate, or glycerol is the energy source and the cells are deprived of amino nitrogen (35, 50). Kadner et al. (50) suggested that methylglyoxal production was due to the depletion of the intracellular phosphate pool and an acidification of the cytoplasm, but there was no direct proof for either of these hypotheses. A more direct mechanism to explain methylglyoxal production involves the relationship of anabolism and catabolism. If there is insufficient ADP to run the normal glycolytic scheme (excess energy), triose phosphates can still be shunted into the non-ATP-generating methylglyoxal shunt (19).

From the observation that methylglyoxal production appears to be a consequence of anabolic and catabolic imbalances, one might argue that energy spilling has the potential to protect bacteria from this toxic substance. This hypothesis is supported by the observation that the potassium efflux channels of *E. coli* are activated by methylglyoxal (29). When *E. coli* was treated with methylglyoxal, there was an increased potassium loss, an increased rate of ATP turnover, and a resumption of normal glycolytic sugar catabolism (less methylglyoxal production). The idea that there might be an inverse relationship between energy spilling and methylglyoxal production is also supported by experiments with *Streptococcus bovis*. When *S. bovis*, a bacterium that has a high capacity to spill energy, was grown under nitrogen limitation in glucose-excess continuous culture, methylglyoxal was never detected and the culture remained viable (91).

## MAINTENANCE VERSUS ENDOGENOUS METABOLISM

When bacteria are depleted of exogenous substrates, the cells often use endogenous materials as an energy source. The terms "endogenous metabolism" and "maintenance energy" have often been used interchangeably (23), but there is evidence that the energetics are not the same. Dawes (23) believed that "maintenance" and "endogenous metabolism" were synonymous, because both processes were related to an "an energized membrane state," but this assumption ignores that fact that many bacteria (e.g., streptococci) often let their membrane potentials decrease as soon as exogenous substrates are depleted. In streptococci, survival is more closely associated with the presence of a phosphoenolpyruvate pool. Phosphoenolpyruvate serves as a means of reactivating the phosphotransferase system of transport (PTS) when exogenous sugar is again available (121).

The cellulolytic bacterium *Fibrobacter succinogenes* lacks a PTS (33, 64) and uses glycogen reserves to sustain the $\Delta\Psi$ that is needed for sodium-driven cellobiose transport, but the maintenance rate of growing cells was at least threefold higher than the endogenous rate of starving cells (127). As the cells starved, the endogenous rate declined and viability did not decrease significantly until the endogenous rate was 20-fold lower than the maintenance rate. The intracellular potassium concentration may be at least partially responsible for the difference between maintenance and endogenous metabolism. Growing cells had a potassium concentration that was at least twofold greater than that of starving but still viable cells (127).

The confusion about maintenance and endogenous metabolism has clouded our understanding of bacterial growth energetics. When dilution rates are decreased to very low values and the drip of medium into the culture vessel becomes very slow, the "continuous" cultures become "discontinuous." To counteract the problem of discontinuous growth, Van Verseveld et al. (125) developed a "recycling fermentor" that operated in a continuous fashion at very low dilution rates, but the growth kinetics were abnormal. Because $Y_{MAX/GLUCOSE}$ was lower than the actual $Y$ and the $m$ coefficient was much lower than values derived at higher dilution rates, the authors concluded that the Pirt-type (85, 86) calculations of maintenance were no longer valid. The inherent difference between maintenance and endogenous metabolism seem a more fitting explanation than a variable maintenance per se.

Since maintenance energy and endogenous metabolism are not synonymous, it is quite possible that a bacterium would have a high maintenance rate and a low endogenous rate, or even vice versa. Maintenance should be used only to define growth when most of the cells in the population are capable of growing. Endogenous metabolism should be defined as a state when no net growth is possible and should not be confused with cryptic growth (cannibalism).

## APPLICATIONS

In traditional industrial fermentations, cell production diverts carbon flow from useful end products (alcohol, solvents, methane, antibiotics, etc.), and under these conditions energy spilling could be a desirable characteristic. When enzymes or other proteins are the output, however, the efficiency of ATP utilization can be critical. If ATP is diverted from protein synthesis to spilling reactions, the yield of protein will decrease. Given the fact that many bacteria (batch cultures) have $Y_{ATP}$ values that are less than one-half of the $Y_{ATP/MAX}$, there is considerable potential for increasing the efficiency of enzyme production by reducing the magnitude of energy spilling.

Ecological models of bacterial growth have generally concentrated on the relationship between substrate concentration and $\mu$ (14, 89, 90), but bacterial biomass production has in nearly all cases been based on empirical yield coefficients. Robinson and Tiedje (90) noted that the yield of bacterial cultures "may be influenced by the growth rate history of the inoculum," but their model did not have specific accommodations for maintenance energy or energy spilling. In a recent model of ecosystem energetics, Ohtonen (80) used a "metabolic quotient (respiration/biomass)" for "calculating the microbial biomass' and noted that a different "calibration" was needed for each soil type.

Ruminant animals depend upon the ruminal microbial ecosystem to transform feedstuffs into useful fermentation products, and microbial protein is the primary amino acid source for ruminant metabolism. Despite the fact that the efficiency of microbial growth in the rumen can have a dramatic impact on the economics of milk and meat production (78), the National Research Council Recommendations of the National Academy of Sciences still use a constant growth yield for ruminal bacteria (71). A kinetic model of ruminal microbial growth indicated that ruminal bacteria devote 20 to 40% of their ATP on maintenance and can spill as much as 18% of their energy (99). When the model was validated by independent studies of animal performance, it became apparent that ruminant diets can be better formulated to (i) increase the efficiency of bacterial growth in the rumen, (ii) decrease feed costs, and (iii) significantly improve the economics of ruminant production (32, 103).

## CONCLUSIONS

When bacteria are limited for energy sources, the free energy change of catabolic reactions is generally tightly coupled to the anabolic steps of cellular biosynthesis, and total energy flux can be partitioned into growth and maintenance functions. If growth is limited by nutrients other than energy, however, bacteria can spill ATP in reactions that cannot be readily categorized as maintenance per se. Recent work indicated that bacteria utilize futile cycles of ions through the cell membrane as a means of hydrolyzing ATP. The mechanism and magnitude of membrane-mediated ATP dissipation is dependent on the physiological state of the bacterium (ATP concentrations, intracellular and extracellular concentrations of ions, and the presence of specific carriers or channels in the cell membrane). Further work is clearly needed to delineate the precise pathways and regulation of these cycles. Energy spilling can be regarded as an energetically wasteful aspect of microbial growth, but it is probably not a fortuitous act. Energy spilling may be a mechanism of rapidly reinitiating growth or protecting cells from potentially toxic schemes of sugar metabolism (e.g., methylglyoxal).

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